GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: Global Institute for Research & Education)
www.gifre.org

EFFECT OF CELL-FREE CULTURE FILTRATES OF BIO-CONTROL AGENTS ON THE SPORE GERMINATION AND INFECTION BY *PHYTOPHTHORA INFESTANS* CAUSING LATE BLIGHT OF POTATO

Chandrakala, A., Chandrashekar, S. C., Jyothi, G. & Ravikumar, B. M.
Department of Plant Pathology, University of Agricultural Sciences, GKVK, Karnataka, Bangalore-560 065, India.

Abstract

Potato ranks fourth as the most important food crop in the world. The crop is known to suffer from many diseases among which late blight is more devastating. Therefore, culture filtrates of bio-control agents, (*Trichoderma virens*, *Trichoderma viride*, *Paecilomyces lilacinus* and *Pseudomonas fluorescens*) were tested for their effectiveness on the spore germination and infection *in-vitro*, as fungicides usage leads to more production cost, besides development of fungicide resistance. *Trichoderma virens*, *Trichoderma viride* and *Pseudomonas fluorescens* culture filtrates showed no spore germination at all the dilution ratios of culture filtrate (CF) and sterile distilled water (SDW) after 48 hours of incubation. In detached leaf assay for infection studies *T. virens*, *T. viride* and *P. fluorescens* culture filtrates inhibited infection by *P. infestans* at all the dilution ratios. *Trichoderma virens* culture filtrate completely inhibited infection in green house grown potato plants. *T. viride* culture filtrate showed 45% of the plant infection.

***Key Words*:** Bio-control agents, Spore germination, Culture filtrate (CF), Infection

Introduction

Potato (*Solanum tuberosum* L.) is an important food crop of the world. This is next only to rice, wheat and maize. It is used as vegetable, stock feed and in industries for manufacturing starch, alcoholic beverages and other processed products. It provides essential body building substances such as proteins, vitamins, minerals (P, Ca, Mg, K, Fe, S, Cl). But potato cultivation is constrained by several biotic factors such as Early blight, late blight, wart, black scurf, charcoal rot, powdery scab, *Fusarium* wilt, *Verticillium* wilt, *Sclerotium* wilt, common scab, Brown rot, soft rot, potato virus X, potato virus Y, crinkle mosaic, potato acuba mosaic virus and witch's broom, of which late blight caused by *Phytophthora infestans* (Mont.) de Bary is considered the most important, highly devastating disease (Fry and Goodwin, 1997) which has become endemic in potato growing areas, with an incidence ranging from 50-100 per cent (Anon., 2010). It can result into crop failures in a short period if appropriate control measures are not adopted. Hence, use of fungicides form one of the major component in disease management. But, regular use of fungicides involves additional production costs and adverse environmental hazards, besides development of resistance against fungicides by pathogens. In recent years, use of antagonistic microflora for control of pathogen is gaining importance. Hence in present investigation, culture filtrate has extracted from bio-control agents to test against *Phytophthora infestans* causing potato late blight disease.

Material and Methods

Collection of the Sample and Isolation

For the isolation of *Phytophthora infestans* from late blight infected potato leaves (preferably having one lesion only) were collected from Chikkaballapur potato growing field. For isolation of *Phytophthora infestans* method followed by Tumwine *et al*. (2000) was followed. The collected samples were surface sterilized with 70% alcohol to get rid of secondary micro organisms. Took the washed tuber with water and peeled –off potato skin with knife, surface sterilized with 70% alcohol. Tuber was sliced to about ±0.5 cm thickness with flamed knife and placed two slices in a petriplate containing blotter paper. Small leaf pieces from the lesion margin containing some dead and some living tissue were inserted in between potato slices with the help of sterile forceps (40% dead and, 60% living). The inoculated potato slices were incubated at 19±1° C for 6-7days. After 6 days of incubation the mycelium had grown through the tuber slice. A little plug of mycelium was transferred with a needle on to V8 agar or sterilized potato slice in sterilized Petri plates containing filter paper.

Preparation of Culture Filtrate (CF) Of Fungal Antagonists and Bacterial Antagonist

Mycelial disks of each antagonistic micro-organism grown on PDA was separately inoculated into 100 ml flasks containing potato dextrose broth and incubated at 25 to 29°C for 15 days. The cultures were then filtered through Whatman filter paper. Then culture filtrates were used for germination test at different concentrations (Doustmorad Zafari *et al.*, *s*2008).

Bacterial Antagonist

The bacteria were cultured in 250ml flask with 50 ml nutrient broth on a rotary shaker at 200 rpm for 24 h at 28°C. The cells were harvested by centrifugation at 10,000 rpm for 10 min and supernatants were filtered through Whatman membrane (2.4 µM) (Katsumi Akutsu *et al.*, 1993).

Spore Germination Test

Hanging drop technique (John Tuite, 1969) was followed to study the spore germination with different antagonist culture filtrates and chemical DCB. Spore suspension was prepared from 8-9days old culture that was grown on surface sterilized potato slices in different ratios of culture filtrate and sterile distilled water of bio-control agents (Table 1) [no dilution (pure culture filtrate), 2(CF):1(SDW), 1(CF):1(SDW), 1(CF):2(SDW), 1(CF):5(SDW)]. One drop of spore suspension was placed at the centre of the cover slip and wax was smeared around the cavity of the slide, then placed cavity slide on the cover slip and reversed the cavity slide. Those cavity slides were placed in Petri-plates lined with moist filter paper and incubated at 10±1˚ C. Three replications were maintained for each treatment. Slides were examined 24, 36 and 48 hours after incubation.

The total number of spores and the number of germinated spores were recorded in five microscopic fields under 10X objective. The germination percentage of spores with different concentrations of culture filtrate of antagonists was calculated using the following formula.

$$\text{Germination percentage} = \frac{\text{Number of spores germinated}}{\text{Total number of spores}} \times 100$$

Table 1. List of antagonists evaluated against *Phytophthora infestans*

Sl. No.	Antagonists
1	*Trichoderma viride*
2	*Trichoderma virens*
3	*Paecilomyces lilacinus*
4	*Pseudomonas fluorescens*

Detached Leaf Assay

Fully expanded leaflets were chosen from plants, which were detached from green house grown plants of 6 weeks age and were selected so that all leaflets were of similar age and size. Leaflets were placed abaxial side up on moistened filter paper/moistened absorbent cotton in glass Petri dishes, and each was inoculated with a 25-µl drop of sporangial suspension which was prepared in sterile distilled water (SDW) for control and with culture filtrate of antagonists in different ratio of culture filtrate and SDW. A single leaflet was used as an experimental unit. Leaflets were then placed in an incubator at 18°C with a 16-hour photoperiod and examined 24 h after inoculation and then every 24 h until 7 days post-inoculation for infection (Jeffrey Miller *et al*., 1998).

Whole Plant Assay in Green House Conditions

Potato plants which were grown in green house conditions were sprayed with sporangial suspension (10^5 sporangia per ml) of *P. infestans*. Plants were covered with polythene bag to create humidity. After one hour and 30 minutes plants were sprayed with culture filtrates of *T. viride* and *T. virens*, except control plants. Again plants were covered with polythene bag for 2 days, on 3rd polythene bags were removed for half a day and again plants were covered. On 5th day observed for results (Stephan *et al*., 2005).

Table 2. Disease scale for scoring late blight of potato

Scale	Number of leaf lets affected	Percentage of plant affected (%)
0	No symptoms	0
1	2-3 leaf lets	25
2	4-5 leaf lets	45
3	6-7 leaf lets	65
4	8-9 leaf lets	85
5	10-11 leaf lets	100

Results and Discussion

***In Vitro* Evaluation of Culture Filtrates of Antagonistic Microflora on Spore Germination**

The cell free culture filtrates of *Trichoderma virens, T. viride* and *Pseudomonas fluorescens* showed no spore germination at all the dilution ratio of CF and SDW after 48 hours of incubation (Table 3 and Fig. 1). Abbas El-Hasan *et al.*, (2009) reported that production of viridiofungin A (VFA) in culture filtrate of *T. harzianum* isolate T23 prevented germination of *Phytophthora infestans* sporangia. Chamber and Scott, (1995) stated that *Trichoderma hamatum* cultures and *Gliocladium virens* in culture filtrate inhibited growth of *Phytophthora cinnamomi* and *Phytophthora citricola*. Cell

free culture filtrates of both *T. viride* and *G. virens* also significantly inhibited the growth of *Rhizoctonia solani*, with effect increasing as the concentration of the filtrates in the culture media increased (Nahed Haikal, 2008). Rajeswari *et al*., (2006) observed that *Pseudomonas fluorescens* as highly effective in reducing the sporangial germination of *P. infestans*. Diby Paul and Sarma, (2006) also showed that metabolites of *P. fluorescens* strain, IISR-6 completely inhibiting the sporangial production in *Phytophthora capsici*.

Whereas undiluted cell free culture filtrate of *Paecilomyces lilacinus* showed zero per cent germination, but at 1:2 and 1:5 dilution ratio *P. lilacinus* showed 12.18 per cent and 20.02 per cent spore germination respectively after 48 hours of incubation (Table 3). Adebola and Amadi, (2010) observed that culture filtrate of the test fungus *Paecilomyces lilacinus* also inhibited the growth of *Phytophthora palmivora*.

***In Vitro* Evaluation of Culture Filtrates Of Antagonistic Microflora On Infection**

Trichoderma virens, *Trichoderma viride* and *Pseudomonas fluorescens* (Fig. 2a, 2b, 2c) culture filtrates inhibited infection by *P. infestans* at all the dilutions ratio in *in-vitro* bioassay (Table 3). Chamber and Scott, (1995) observed that *Trichoderma hamatum* and *Gliocladium virens* prevented *P. cinnamomi* and *P. citricola* from causing infection symptoms on micropropagated shoots of chestnut in an *in vitro* excised shoot bioassay for bio-control. The effectiveness of culture filtrates of *T. virens* and *T. viride* on infection of potato plants by *P. infestans* was studied using whole plant assay method. *T. virens* completely inhibited infection. *T. viride* showed 45% of the plant infection (Fig. 3).

Table 3: *In vitro* evaluation of culture filtrates of bio- agents against *Phytophthora infestans*

Sl. No.	Treatment	Dilution ratio of CF and SDW	Spore germination (%)			Infection on detached leaf
			24 hrs	36 hrs	48 hrs	
1	***Trichoderma virens***	Un-dilution	00.00	00.00	00.00	NI
		2:1	00.00	00.00	00.00	NI
		1:1	00.00	00.00	00.00	NI
		1:2	00.00	00.00	00.00	NI
		1:5	00.00	00.00	00.00	NI
2	***Trichoderma viride***	Un-dilution	00.00	00.00	00.00	NI
		2:1	00.00	00.00	00.00	NI
		1:1	00.00	00.00	00.00	NI
		1:2	00.00	00.00	00.00	NI
		1:5	00.00	00.00	00.00	NI
3	***P. lilacinus***	Un-dilution	00.00	00.00	00.00	NI
		2:1	00.00	00.00	00.00	NI
		1:1	00.00	00.00	00.00	NI
		1:2	19.75	24.80	31.18	I
		1:5	21.32	30.85	44.89	I
4	***P. fluorescens***	Un-dilution	00.00	00.00	00.00	NI
		2:1	00.00	00.00	00.00	NI
		1:1	00.00	00.00	00.00	NI
		1:2	00.00	00.00	00.00	NI
		1:5	00.00	00.00	00.00	NI
5	**Control**	SDW	92.85	97.50	99.06	I
S. Em±			0.144	0.121	0.155	
CD @ 5%			0.553	0.462	0.594	
CV (%)			1.287	0.819	0.922	

Infection = I , No infection= NI

***Phytophthora infestans* culture on potato slice**

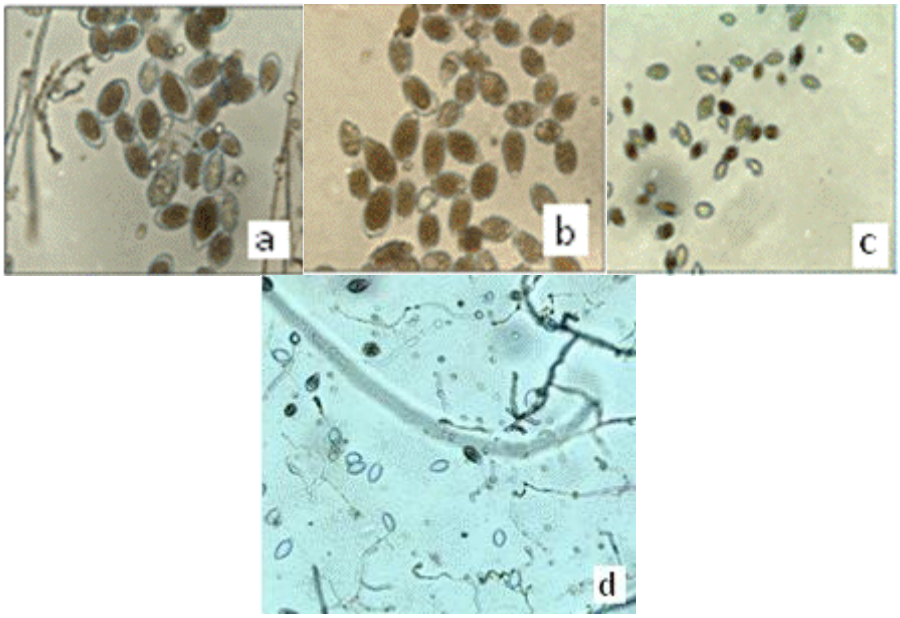
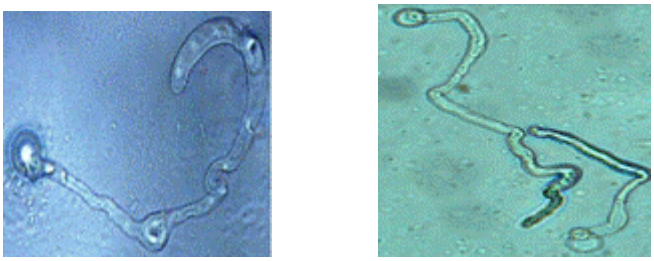
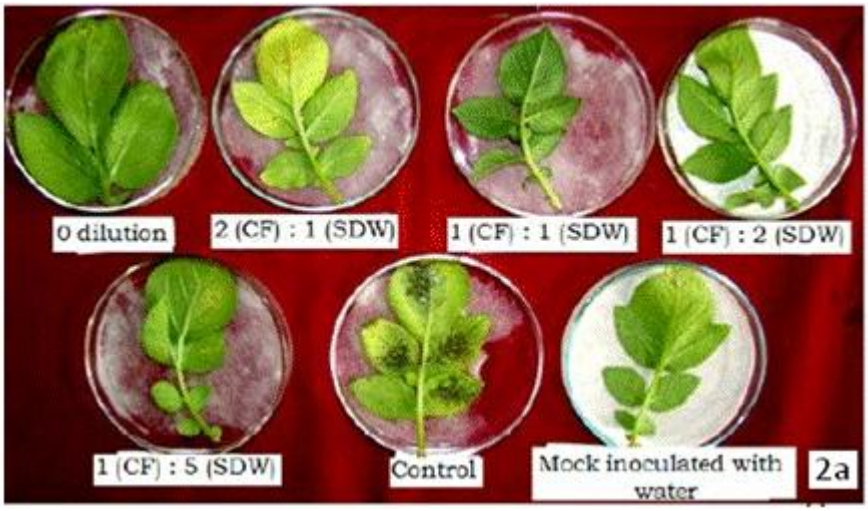


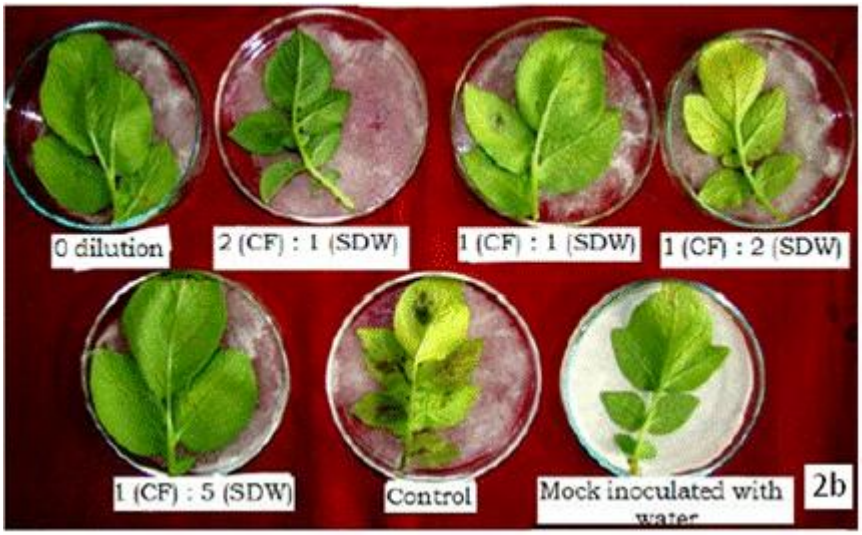
Figure 1: Effect of culture filtrate of antagonists on spore germination
a=*T. virens*, b=*T. viride*, c =*P. fluorescens*, d= Control



Enlarged view of germinated zoospores



Trichoderma virens



Trichoderma viride

Figure 2a, 2b: Effect of cell free culture filtrates of antagonists against *P. infestans*

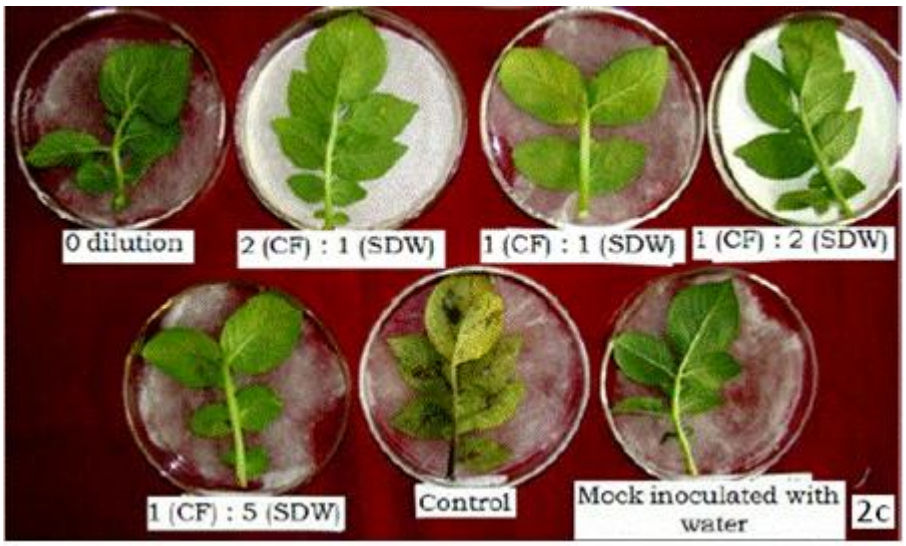


Figure 2c: Effect of cell free culture filtrates of *Pseudomonas fluorescens* against *Phytophthora infestans*

Figure 3: Effect of culture filtrate of *T. virens* and *T. viride* against *Phytophthora infestans* on whole potato plants

Conclusion

It is concluded from the experiment that culture filtrates of antagonists like *Trichoderma virens*, *Trichoderma viride* and *Pseudomonas fluorescens* having the potential of preventing or inhibiting the germination of *Phytophthora infestans* sporangia causing late blight of potato and also preventing the infection from *Phytophthora infestans* in *in-vitro* conditions.

Acknowledgement

I gratefully acknowledge Mr. S. C. Chandrashekar Associate Professor, Department of Plant Pathology, GKVK, Bengaluru, chairman of my advisory committee for his inspiring guidance at every step, critical comments, and constant supervision. I wish to express my earnest and profound sense of gratitude to Dr. K. T. Rangaswamy, Associate Professor my advisory committe member, Department of Plant Pathology, GKVK, Bengaluru for his constant encouragement, support throughout the course of investigation. I am thankful to the staff members of the National Bureau of Agriculturally Important Insects, Dr. B. Ramanujam, Dr. S. Sriram and Dr. R. Rangeshwaran for providing bio-control agents for my research work.

References

Abbas El-Hasan, Walker Frank, Schone Jochen and Buchenauer Heinrich, 2009. Detection of viridiofungin A and other antifungal metabolites excreted by *Trichoderma harzianum* active against different plant pathogens. *European J. Pl. Pathol.*, **124**(3): 457-470.

Adebola, M.O., and J. E. Amadi, 2010. Antagonistic activities of *Paecilomyces* and *Rhizopus* species against the cocoa black pod pathogen (*Phytophthora palmivora*). *African Scientist*, **11**(4): 467-470.

Anonymous, 2010. *Annual report*, Central Potato Research Institute, Shimla, India, 125pp.

Diby Paul Y. R. Sarma, 2006. Antagonistic effects of metabolites of *Pseudomonas fluorescens* strains on the different growth phases of *Phytophthora capsici*, foot rot pathogen of black pepper (*Piper nigrum* L.). *Arc. Phytopathol. Protect*., **39**(2): 113-118.

Doustmorad Zafari, Mehdi Mehrabi Koushki and Eidi Bazgir, 2008. Bio-control evaluation of wheat take-all disease by *Trichoderma* screened isolates. *African J. Biotech*., **7**(20): 3653-3659.

Fry, W.E., and Goodwin S.B. 1997. Re-emergence of potato and tomato late blight in the United States. *Plant Disease*, **81:** 1349–1357.

Jeffrey S. Miller, Dennis A. Johnson, and Philip B. Hamm, 1998. Aggressiveness of Isolates of *Phytophthora infestans* from the Columbia Basin of Washington and Oregon. *Phytopathology*, **88**(3): 191-197.

John Tuite. 1969. *Plant pathologiocal methods: Fungi and bacteria*, 229pp. University of Minnesota, US.

Katsumi Akutsu, Akiko Hirata, Michiko Yamamoto,Satoshi Okuyama and Tadaal Kihibi, 1993. Growth inhibition of *Botrytis* spp. by Serratia marcescence isolated from tomato phylloplane. *Ann. Phytopath. Soc. Japan*, **59**: 18- 25.

Rajeswari, E., K. Chitraa; K. Seetharamana; V., 2006. Exploiting medicinal plants and phylloplane microflora for the management of grapevine downy mildew. *Res. J. Agri. Biol. Sci*., **2**(6): 262-267.

Stephan, D, A. Schmitt, S. Martins Carvalho, B. Seddon and E. Koch, 2005. Evaluation of biocontrol preparations and plant extracts for the control of *Phytophthora infestans* on potato leaves. *European J. Plant Pathol.*, **112**(3): 235-246.

Tumwine, J., H.D. Frinking and M.J. Jeger, 2000. Isolation techniques and cultural media for *Phytophthora infestans* from tomatoes. *Mycologist*, **14**: 137-139.